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PROGRESS REPORT

This progress report covers a period of 10 years of work supported by the Office of the Surgeon General, The United States Army. Because of the broad scope of the research, the report will summarize progress made in a meritive fashion rather than to detail specific achievements. The project has concerned itself with "Metabolic Disorders and Therapeutic Approaches in Renal Failure". The report will be divided into three phases.

1) Metabolic Disorders, 2) Treatment of Renal Failure, and 3) Homotransplantation of Tissue with particular reference to the kidney.

1) Metabolic Disorders: Both in the uremic patients and in uremic animals we have endeavored to elaborate the metabolic defect which results in kidney failure. Early in our course we noted that patients with renal failure improved significantly if they were dialyzed against a bath fluid containing urea, suggesting that urea alone was not the only cause for the morbid state. Furthermore, we demonstrated that liver slices incubated in 100 millimoles per liter of urea showed no inhibition of pyruvate uptake or glucose production. Although several years were spent in attempts to utilize tissue slices for this, the tissue slice itself was unsatisfactory as a purely in vitro system. We were, however, able to show that increasing concentrations of urea beginning at .05 molar did inhibit monamine oxidase activity of rabbit kidney slices acting on a pyramine substrate. This work was of interest since the inhibition was a diphasic one, decreasing with increasing concentrations and suggesting possible polymerization of urea at higher concentrations. The significance of this interpretation relates to one of the earliest observations made in our work when we were able to show that more urea could be removed from chronic uremic patients than could be accounted for by the decrease in blood urea nitrogen concentration, suggesting that urea was perhaps polymerized, or at least was not equally distributed throughout the total body water. This very early observation has been confirmed in a number of ways in recent years. We were able to show

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in rats made acutely uremic, there was no demonstrable defect in the formation of liver glycogen nor the absorption of glucose in the intestine. In rats with chronic uraemia we could demonstrate a marked decrease in glucose uptake from intestine and a marked decrease in liver glycogen as well as impairment of glucose utilization following a glucose load. This is of interest in view of recent evidence in patients that a similar effect may occur in uremia.

Patients with acute uremia showed decreased survival of red blood cells which were infused and whose half-life was followed by the use of isotope tagging. This decreased survival was improved following dialysis with the artificial kidney and disappeared within five days after the implantation of a normal kidney. Since the defect in some way resembles the so-called 'storage defect' and since this could be improved in vitro by Inosine, we attempted to infuse Inosine into patients with chronic renal failure and study the red blood cell defect particularly as manifested by abnormalities in the intracellular concentration of red cell sodium potassium. The infusion of Inosine into uremic patients did indeed reverse this defect but the hyperuricemia which resulted from metabolism of the purine moiety of Inosine to uric acid, required hemodialysis. Other intracellular defects in chronic uremia have also been studied. We showed some time ago that in chronic renal failure rapid dialysis with the artificial kidney might return serum bicarbonate rapidly to normal but that since bicarbonate did not cross the cell membrane rapidly, hyperventilation continued and a respiratory alkalosis was substituted for the metabolic acidosis.

Current work is concerned with the correct measurement of this phenomenon by the use of the measurement of the volume distribution of D₂O.

With the Neurologic Service we studied intensively the neurologic abnormalities in both acute and chronic uremia. It was observed that most of the neurologic abnormalities in chronic uremia were apparently due to a metabolic defect similar to that seen in liver disease. In this connection, a so-called liver flap is not uncommon in uremic patients. Electroencephalographic abnormalities were

common and these could be roughly quantitated by the response of the electroencephalogram to photic stimuli. Most important was the fact that focal neurologic abnormalities were transient and appeared to be not the result of focal organic central nervous system lesions. Furthermore, hypocalcemia was rarely the cause of any of the neurologic manifestations observed.

Extensive work on cardiac arrhythmias occurring in renal failure have been done. Cardiac arrhythmias can be produced by rapid changes of extracellular pH, by raising the serum sodium, or by dropping the serum potassium. Isotope dilution studies have shown that there is a correlation between these arrhythmias or the changes in the electrocardiogram and the total body potassium or the extracellular level. It appears that the electrocardiogram and the sensitivity to cardiac arrhythmias may be a function of the relative concentrations of sodium and potassium across the cell membrane.

During the course of the above studies with isotope dilutions we were able to elicit the fact that typically in acute renal failure, extracellular fluid volume is expanded, probably by the production of metabolic water from the catabolism of fat. In addition, in both acute and chronic renal failure serum citrate levels may be markedly elevated, possibly contributing to the so-called unidentified acid fraction about which there has been much speculation.

A fortuitous consequence of this work on body fluid volume were the observations that in man, renoprival hypertension does not exist. Our ability to maintain people with no renal function whatever in relatively good states of health enabled us to observe that in four patients in whom a single kidney had been removed accidentally, hypertension did not occur unless the volume of extracellular fluid was increased. Salt and water deprivation in these individuals returned blood pressure levels to normal. The importance of this observation in man when considered in the light of the numerous claims from animal experiments that the kidney has an important depressor function, is obvious.

2) Treatment of Renal Failure:

A major function of our work in the treatment of acute renal failure has been concerned with the artificial kidney. The rotating artificial kidney, first described by Kolff, was modified to become a practical clinical tool in this laboratory. During the course of its development and use we had occasion to train a number of Army personnel in its use. Work with this apparatus in the Korean War contributed importantly to knowledge both of hemodialysis and the treatment of acute renal failure. The importance of the use of the artificial kidney in the prophylactic treatment of acute renal failure is too well known to require emphasis. However, it is of some interest that one week ago, dialysis number one thousand was done in this hospital. It would not be incorrect to say that the vast majority of these dialyses were made possible by support from the office of the Surgeon General and knowledge derived from this has contributed importantly to the treatment of both acute and chronic renal failure in this country and elsewhere.

In 1953 the investigator and Dr. Marcel Legrain published our experience with a new technique with peritoneal dialysis using a plastic tube inserted into the peritoneum through a simple paracentesis technique. This simplified technique in a somewhat modified form is now in wide-spread use. More extensive application necessitated the production of commercial rinsing fluid. In 1957 we reported the removal of potassium by passing blood from the uremic patient over an ion exchange column. Because of platelet and white cell destruction, however, this technique was not universally applicable. However our work at a later date with the fine mesh ion exchange resin, (Kay-exalate) particularly facilitated by the ingestion of sorbitol, proved to be an extremely practical measure for the removal of potassium from gastro-intestinal secretions and is now widely utilized. Work with Dr. William Looney in this period demonstrated that both the artificial kidney and ion exchange resins were effective means of ridding the body of radioactive calcium and strontium 90.

Experience with the use of indwelling vascular cannulae for chronic hemodialysis has been obtained. As demonstrated by Scribner and his colleagues, this is now an effective means for maintaining useful life in patients with severe chronic renal failure. Of particular interest in our experience is a patient, both of whose kidneys were removed 7 months ago, and who has been maintained since that time by chronic hemodialysis. Observations on the response of his malignant hypertension to bilateral nephrectomy and dehydration have confirmed our earlier observations about the response of hypertension to salt and water restriction in the absence of existing renal tissue. We have maintained a number of patients on chronic peritoneal dialysis, four of these have been maintained at home through the use of dialysis through an indwelling, surgically placed, plastic conduit. Recently a device has been given preliminary trial which will automatically cycle dialyzing fluid and should enable the patients to be dialyzed at home while they sleep. Problems of infection and fibrosis, however, remain to be overcome.

3) Homotransplantation of Tissue:

The emphasis in our study of tissue transplantation has been on the transplantation of the kidney as an approach to the therapy of chronic renal failure. Successful transplantation of the kidney depends upon the knowledge of immunity in tissue transplantation, generally, and consequently, both laboratory work and clinical investigation have been concerned with problems both of basic immunologic concept as well as the transplantation of skin. Our achievements in the field of clinical transplantation have been as follows: 1954, the first successful transplantation of a kidney between identical twins. In 1959, the first successful transplantation of kidney between nonidentical twins. In 1962, the first successful transplantation of a totally unrelated cadaver kidney that has survived at the present time for 15 months. More than 60 human renal homografts have been performed to date. Our first successful transplantation between nonidentical twins was accomplished with the aid of subtotal body irradiation. In this case it was demonstrated for the first time that partial tolerance for a kidney might be obtained and that when the rejection process appeared in the kidney it might be aborted or reversed by the use of cortico-steroids

and further x-irradiation. This concept of partial tolerance and the ability to "abort" a rejection response has been of paramount importance, both in our subsequent work and in the work of others. In the patient in whom a cadaver kidney was transplanted, 7 separate and distinct episodes of abortive rejection occurred which were reversed with the use of corticosteroids and immuno-suppressive therapy. Our recent work based largely on the studies of Doctors Murray, Calne and others in our laboratory has utilized the use of immuno-suppressive therapy in the form of drugs. At the present time 5 patients are alive and in good health with kidneys transplanted from other than twins. The survival time varies from 1 month to 7 months.

In addition, we have been interested in skin transplantation. We have demonstrated a sharing of transplantation antigens in humans. We have demonstrated also that following the rejection of a skin graft the patient becomes immune to the leukocytes of the donor and exhibits a delayed hypersensitivity to the interdermal injection of these leukocytes. An interesting offshoot of this work has been the fact that patients with disseminated lupus erythematosus showed the same type of skin hypersensitivity to their own leukocytes. In conjunction with the Departments of Surgery and Pathology, we have carried out a detailed analysis of the histology of the rejection of a human skin homograft by the use of daily biopsies. We have shown that humans may develop an accelerated rejection to skin homografts by prior intradermal injection of donor leukocytes.

Doctor Edward B. Hager in our laboratory has shown that dogs may be immunized to canine homografts by the prior injection of isolated platelets from the donor. In addition to this, Doctor Hager has been able to elute antibody from a rejecting kidney to react this with soluble antigen from donor platelets in an in vitro system. His work shows a greater specificity of the antibody for pure donor antigens than it does to other canine antigens similarly eluted, pointing the way to a possible system in which an individual specific test system for antigen and antibody in mammalian homografts may be developed.

Doctor James Nowbray in our laboratory has shown that an alpha-1 glyco-protein found in serum and concentrated in the thymus gland, may markedly prolong the life

of 30% of skin grafts between rabbits, when injected into the recipient prior - and shortly after - the application of the homograft.

We have also been able to demonstrate a specific cytotoxic effect between cells harvested from donor and recipient rabbits following the immunization of one of the pair by two skin homografts from a second. We have developed an in vitro system for the evaluation of circulating antibody, based not on histologic assay, but on a dynamic assay. This involves the principle of measuring the short-circuit current generated by sodium transport, first in frog skin and later in the rabbit colon, and assaying the effect upon this current by injection into blood vessels supplying these tissues of antibody-containing sera and sensitized leukocytes.

The above review of ten years of work impresses the responsible investigator with the fact that much progress has been made. A good bit of this has been a by-product of work stemming from a protocol which was an offshoot of that for which the research application was made. This fact, it seems, attests to the merit of the research grant as a tool lent by an enlightened granting agency to the investigator in such a fashion that the latter is free to develop his research in the direction which seems most profitable as the work progresses.